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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

New chemotherapeutic agents are needed for the improved treatment of breast cancer. In this proposal, we disclose a new approach to the design of anti-cancer drugs. Our method is to synthesize new drug conjugates that incorporate: (i) a specific breast cancer cell -targeting component; (ii) a rapid cell membrane translocating /nuclear localization moiety and; (iii) the capability to counter multi-drug resistance mediated by P-glycoprotein and related cellular efflux pumps. The conjugates are prepared in a few synthetic steps from available components. The goal is to demonstrate a proof-of-concept of the effectiveness of this multi-functional drug hypothesis.

Specific cancer cell-targeted compounds have been prepared from the breast cancer drug doxorubicin. This cytotoxic agent is covalently linked to a synthetic arginine-glycine-aspartic acid peptidomimetic, which recognizes and binds to a_vb_3 integrin. This receptor is overexpressed on the surface of breast cancer metastatic cells and tumors. The design also includes incorporation of the Tat peptide analog, $H_2N[arginine]_7COOH$, as a rapid cell membrane translocation and effective nuclear localization moiety. The new drugs will be evaluated in breast cancer cell-lines *in vitro* and *in vivo* using human breast cancer xenografts in nude mice.

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A. Introduction

In this program, we are examining a new approach to the design of anti-cancer drugs that is directed toward (i) improving cytotoxic action against cancer cells, (ii) reducing unwanted systemic side effects, (iii) counteracting multi-drug resistance, and (iv) targeting and destroying metastatic cells as well as tumors more effectively.

Our plan is to synthesize new drug conjugates that incorporate a specific breast cancer cell targeting component, a rapid cell membrane translocating/nuclear localization moiety, and the capability to counter multi-drug resistance mediated by P-glycoprotein and related cellular efflux pumps. The conjugates will be prepared in a few synthetic steps from available intermediates. The goal is to demonstrate a proof-of-concept of the effectiveness of this multi-functional drug hypothesis.

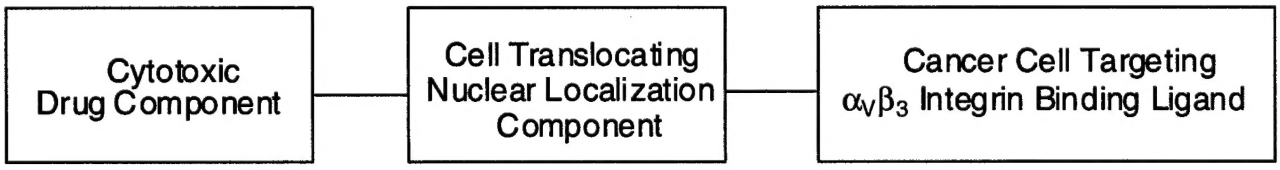
Specific cancer cell-targeted compounds are being prepared from the breast cancer drug doxorubicin. This cytotoxic agent is covalently linked to a synthetic arginine-glycine-aspartic acid peptidomimetic, which recognizes and binds to $\alpha_V\beta_3$ integrin overexpressed on the surface of breast cancer metastatic cells and tumors. The design also incorporates the Tat peptide analog, $H_2N[arginine]_7COOH$, as a rapid cell membrane translocation and effective nuclear localization moiety. Because the targeted conjugates will be rapidly directed into the cell nucleus for efficient cytotoxic effects, the drugs may escape cytoplasmic cleansing, which is mediated by cellular efflux pumps thereby abrogating an important multi-drug resistance mechanism. The new drugs will be evaluated in breast cancer cell-lines *in vitro* and *in vivo* using human breast cancer xenografts in nude mice.

B. Body

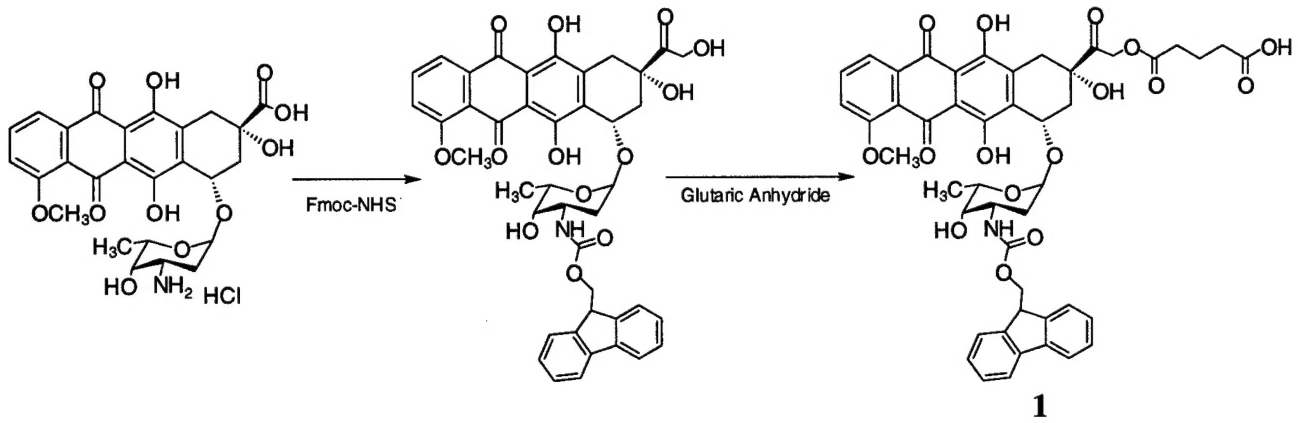
This section describes research accomplishments to date associated with the tasks outlined in the original award application.

Task 1. Synthesize several covalent conjugates utilizing the anti-tumor drugs doxorubicin and paclitaxel, which are linked to a cell translocating/nuclear localizing arginine peptide and a selective breast cancer cell targeting ligand, as well as appropriately linked components as controls **(Months 1-18)**

The three-component conjugates are being assembled according to the arrangement shown below.



Our initial attempts to prepare doxorubicin conjugates utilized the derivative **1** (**Scheme 1**)[1].

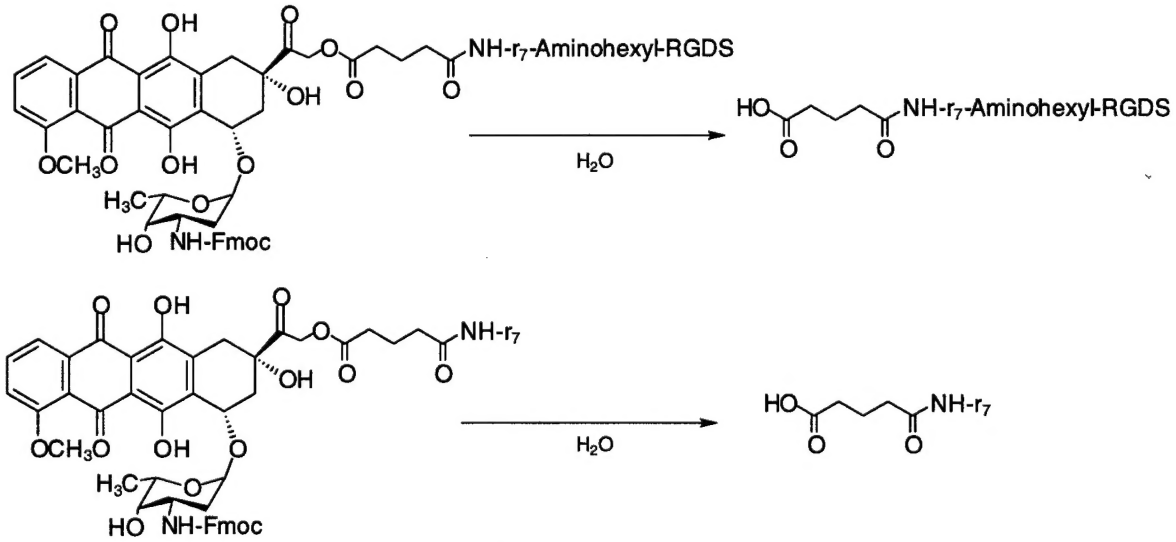


1

Scheme 1

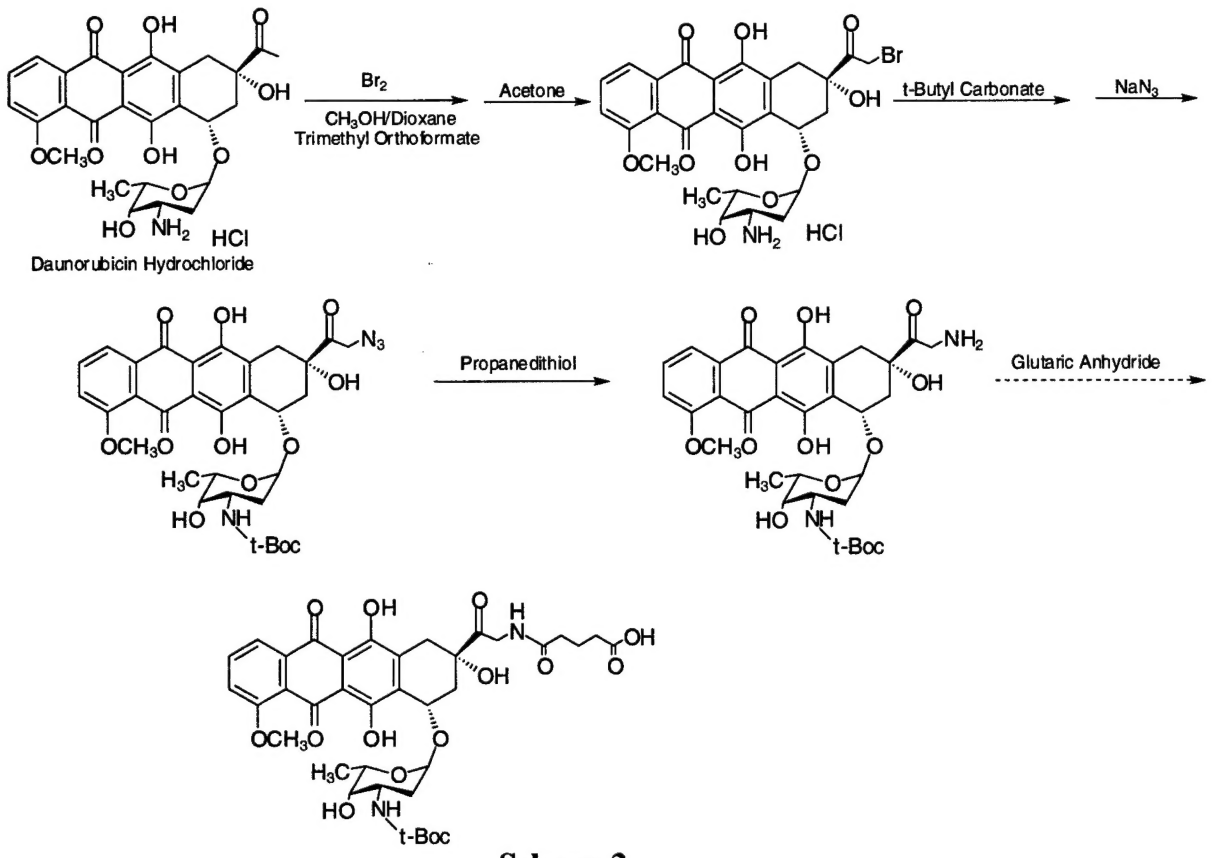
Derivatized doxorubicin **1** was condensed after carbodiimide activation with the cell-translocating peptide, H_2N[D-arginine]$_7$COOH (r_7) and with the peptide H_2N[D-arginine]$_7$CONH-Aminohexyl-RGDS (r_7-Aminohexyl-RGDS), which incorporates the relatively low affinity $\alpha_V\beta_3$ integrin peptide-ligand, arginine-glycine-aspartic acid-serine (RGDS).

Interestingly, both peptide conjugates appear to be unstable in aqueous solution, hydrolyzing readily at the ester bond (**Scheme 2**). This is perhaps the result of the catalytic effect of the multiple guanidine functionalities present in the conjugates[2].



Scheme 2

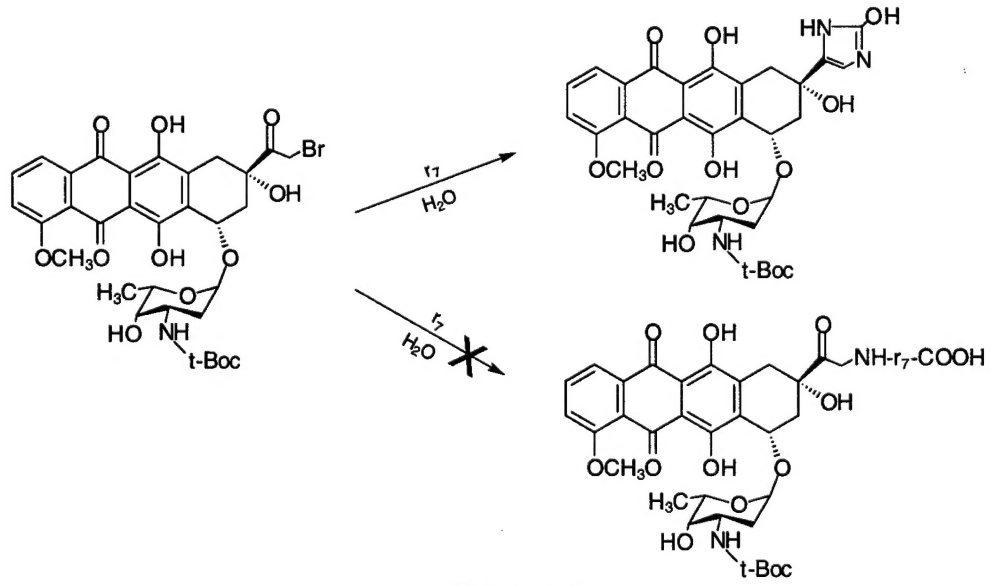
Therefore, we turned our attention to doxorubicin derivatives with an amide at the C-14 position (**Scheme 3**)[3].



Scheme 3

We found this route unsatisfactory overall, perhaps do to the inherent instability of the α-amino ketone functionality.

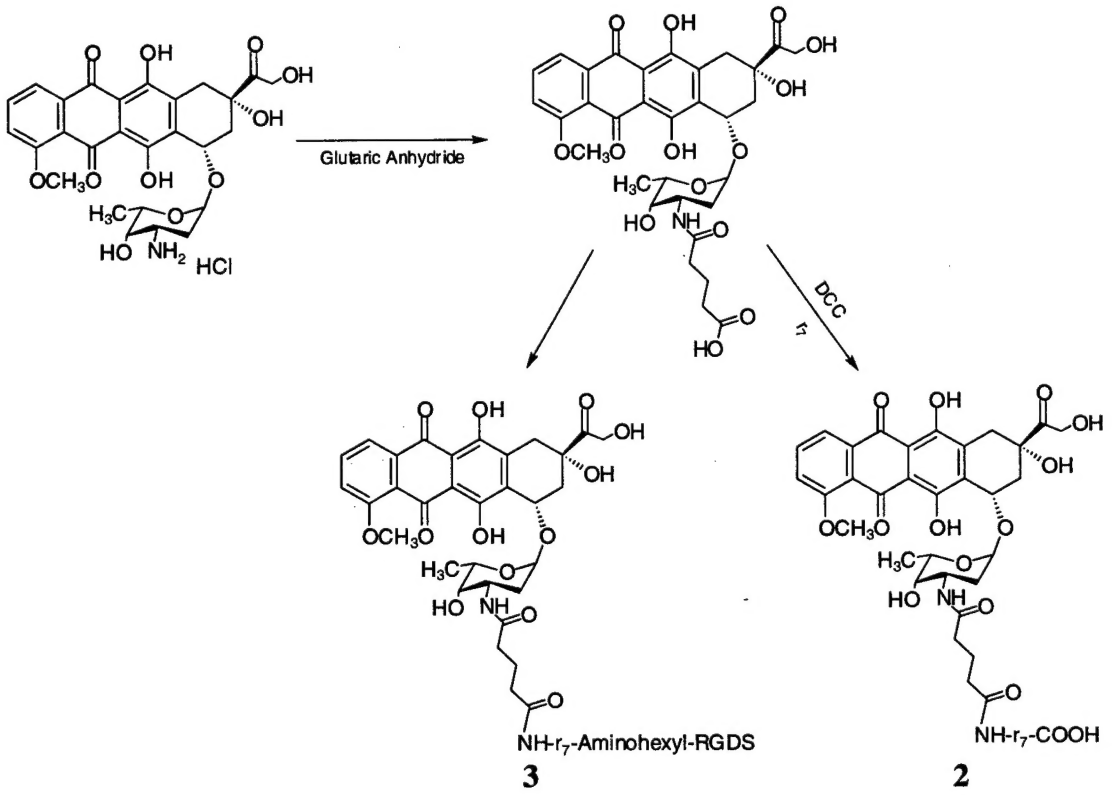
An attempt was made to derivatize r_7 directly with the *t*-Boc-bromo compound prepared above (**Scheme 4**).



Scheme 4

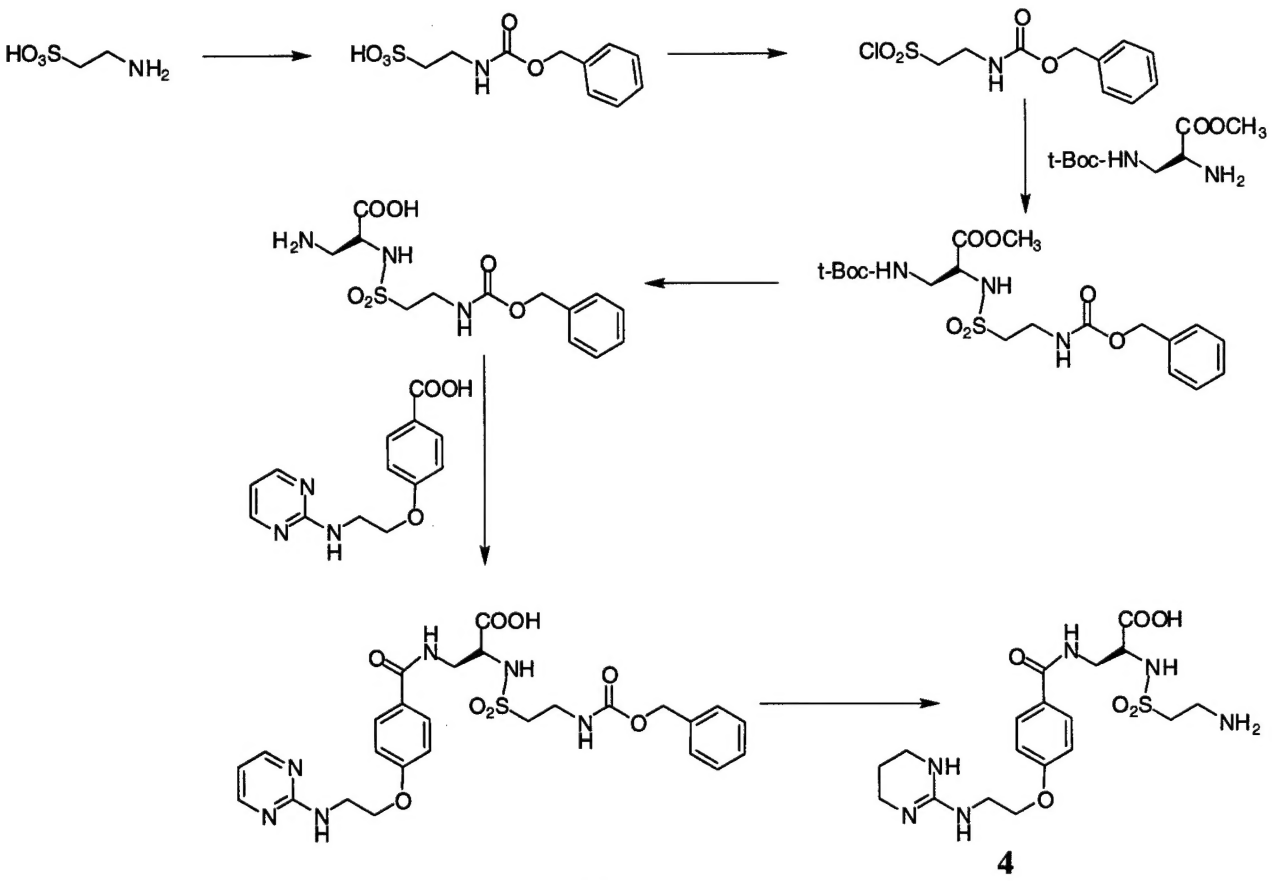
The product isolated seems to be an imidazole derivative. The α-bromo ketone appears to have reacted preferentially with the arginine guanidine functionality rather than the intended peptide terminal amine.

We then turned our attention to doxorubicin conjugates derivatized at the sugar amine (**Scheme 5**).



Scheme 5

Using this route (**Scheme 5**), we have successfully prepared conjugates **2** and **3** and are now ready to begin preliminary cell localization experiments (*Task 2*). Conjugate **2** will be derivatized with the high affinity $\alpha_V\beta_3$ integrin ligand **4** (**Scheme 6**)[4]. Graduate Research Assistant, Jiang Sha, is synthesizing compound **4.**



Scheme 6

Task 2. Establish analytical approaches (confocal microscopy) to monitor the translocation of the doxorubicin conjugates into cells **(Months 9-24)**

With the availability of synthesized conjugates we are on schedule and are now preparing for cell culture experiments.

Task 3. Compare the cytotoxic efficacy of the drug conjugates (*vs.* free doxorubicin and paclitaxel) in human breast cancer and normal breast cell lines **(Months 12-24)**

This task is scheduled for later in the program.

Task 4. Evaluate the efficacy of the conjugates (*vs.* free doxorubicin and paclitaxel) in human breast cancer tumor xenografts in nude mice **(Months 24-36)**

This task is scheduled for later in the program.

C. Key Research Accomplishments

Key accomplishments from Year One of this research are listed below.

- Derivatized doxorubicin derivatives have been prepared.
- Doxorubicin conjugates have been prepared incorporating the [D-arginine]$_7$ cell membrane translocating functionality.
- A doxorubicin conjugate incorporating the [D-arginine]$_7$ cell membrane translocating group coupled to the low affinity $\alpha_V\beta_3$ integrin ligand, RGDS has been synthesized.
- All newly-synthesized compounds have been purified and chemically characterized.
- A high affinity $\alpha_V\beta_3$ ligand is being synthesized for coupling to doxorubicin-r_7.

D. Reportable Outcomes

This program supports graduate research assistant, Jiang Sha, and the results from the research will be incorporated into his dissertation.

E. Conclusions

Research on this effort thus far has provided modified doxorubicin intermediates suitable for attachment to a cell membrane translocating functionality and $\alpha_V\beta_3$ integrin targeting ligands. The resulting conjugates are ready for use in breast cancer cell culture experiments in order to ascertain cytotoxicity as well as selectivity for cancer cells over normal cells.

This research is significant in that it represents the first known examples of cancer chemotherapeutic agents incorporating a drug chemically linked both to a breast cancer-targeting moiety as well as a cell membrane translocating/nuclear localization functionality. The conjugates are expected to show selective targeting to breast cancer cells in preference to normal cells as well as exhibiting enhanced cancer cell cytotoxic effects.

F. References

(1) Nagy, A., Schally, A., Armatis, P., Szepehazi, K., Halmos, G., Kovacs, M., Zarandi, M., Groot, K., Miyazaki, M., Jungwirth, A., and Horvath, J. (1996) Cytotoxic analogs of luteinizing hormone-releasing hormone containing doxorubicin or 2-pyrrolinodoxorubicin, a derivative 500-1000 times more potent. *Proc. Nat. Acad. Sci. USA 93*, 7269-7273.

(2) Haruki, E., Fujii, T., and Imoto, E. (1966) Catalytic hydrolysis of esters by amidines. *Bull. Chem. Soc. Japan 39*, 852.

(3) Bridon, D. P., Leger, R., Huang, X., Milner, P. G., Smith, D., and Ezrin, A. M. Preparation and formulation of long lasting antineoplastic agents, *PCT Int. Appl.*; (Conjuchem, Inc.), 2001; 99 pp, CAS 134:222561.

(4) Hood, J., Bednarski, M., Frausto, R., Guccione, S., Reisfeld, R., Xiang, R., and Cheresh, D. (2002) Tumor regression by targeted gene delivery to neovasculature, *Science*, *296*, 2404-2407.

G. Appendix

Biosketches

Jerald C. Hinshaw, Principal Investigator

Jiang Sha, Graduate Research Assistant

BIOGRAPHICAL SKETCH

Provide the following information for the Principal or Co-Principal Investigators
Follow this format for each person.

NAME	POSITION TITLE
HINSHAW, JERALD CLYDE	Research Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing. Include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Oregon State University, Corvallis, Oregon	BS	1962 - 1966	Chemistry
The University of Utah, Salt Lake City, Utah	PhD	1966 - 1970	Organic Chemistry

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

Research and Professional Experience:

1970-1978 Advanced from Senior Research Chemist to Research Associate, Organic Research Laboratory, Chemistry Division, Research Laboratories, Eastman Kodak Company

1978-1984 Scientist, Research and Development Laboratories, Thiokol Corporation

1980, 1986 Member, Utah Award Committee, Salt Lake Section, American Chemical Society

1981 Visiting Research Associate, University of Utah.

1981-1983 Chairman-Elect, Chairman, Past-Chairman, Salt Lake Section, American Chemical Society

1984-1990 Supervisor, Propellant Research Section, Research and Development Laboratories, Thiokol Corporation

1990-1999 Manager, Energetic Materials Research Department, Research and Development Laboratories,Thiokol Propulsion, Brigham City, Utah.

1996-1999 Member, State Advisory Council on Science and Technology (State of Utah, Governor appointment)

1997,1998 Member, Utah State Governor's Medal for Excellence in Science and Technology Award Committee

1997-1999 Chairman, State Advisory Council on Science and Technology (State of Utah, Governor appointment)

1997-1999 Member, Utah Centers of Excellence Program Advisory Council (State of Utah, Governor appointment)

2/99-7/99 Senior Staff to the Technical Director, Science and Engineering, Thiokol Propulsion, Brigham City, Utah

7/99-11/01 Research Assistant Professor, Department of Medicinal Chemistry, The University of Utah, Salt Lake City, Utah

11/01-current Research Associate Professor, Department of Medicinal Chemistry, The University of Utah, Salt Lake City, Utah

Research Interests:

Synthetic chemistry
Synthesis of bacterial oxidosqualene cyclase inhibitors
Cancer immunotherapy
Targeted drugs
Design and synthesis of small molecule inhibitors of protein-protein signaling
Design and synthesis of fluorescent phosphoinositide probes
Research and technology management.

Honors:

Listed in "American Men and Women of Science"
Listed in "Who's Who in Technology"
Named Outstanding Senior in Chemistry, 1966
National Defense Education Act Title IV Fellow, 1968-1970
Franklin Award, Thiokol Corporation recognition for outstanding technical achievement, 1995

Publications/Patents: J. C. Hinshaw has over 50 publications and patents. Those for 2000-2003 are listed.

A. Ponstsler, A. Silva, A. St. Hilarie, L. Tjoelker, Y. Xu, J. Hinshaw, G. Prestwich, G. Zimmerman, and T. McIntyre "Lysophosphatidic Acid is a transcellular PPARγ Agonist", *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 131-136.

S. Davies, A. Pontsler, G. Marathe, K. Harrison, R. Murphy, J. C. Hinshaw, G. D. Prestwich, A. Hilaire, S. Prescott, G. Zimmerman, and T. McIntyre, "Oxidized Alkyl Phospholipids are Specific, High Affinity PPARγ Ligands", *J. Biol. Chem.*, 2001, **276**, 16015.

J. C. Hinshaw and G. D. Prestwich, "The Design, Synthesis, and Evaluation of Molecules That Enable or Enhance Cellular Uptake: Peptoid Molecular Transporters", *ChemTracts, Organic Chemistry*, 2001, **14**, 391. Commentary on the research by P. Wender, D. Mitchell, K. Pattabiraman, E. Pelkey, L. Steinman, and J. Rothbard, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 13003.

J. C. Hinshaw and G. D. Prestwich, "Pursuit of Optimal Carbohydrate-Based Anti-cancer Vaccines: Preparation of a Multi-antigenic Unimolecular Glycopeptide Containing the Tn, MBr1, and Lewisy Antigens", ChemTracts, Organic Chemistry, 2001, 14, 217. Commentary on the research by J. Allen, C. Harris, and S. Danishefsky, *J. Amer. Chem. Soc.*, 2001, **123**, 1890.

J. C. Hinshaw, D. W. Doll, R. J. Blau, G. K. Lund, " Metal Complexes for Use as Gas Generants," U.S. Patent 6,241,281, issued June 5, 2001.

R.B. Wardle, R.M. Hajik, J.C. Hinshaw, T.K. Highsmith, "Process for the Large-Scale Synthesis of 4,10-Dinitro-2,6,8,12-Tetraoxa-4,10-Diazatetracyclo[5.5.0.$0^{5,9}0^{3,11}$]dodecane," U.S. Patent 6,107,483, issued August 22, 2000.

J. C. Hinshaw, D. W. Doll, R. J. Blau, G. K. Lund, " Metal Complexes for Use as Gas Generants," U.S. Patent 6,039,820, issued March 21, 2000.

G. D. Prestwich, F. S. Buckner, J. C. Hinshaw, "Methods Related to Steroid Metabolism of Parasites and Mycobacteria, and Treatment of Parasite and Mycobacterial Infections with an Oxidosqualene Cyclase Inhibitor", U.S. Patent Application filed June 16, 2000.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.

NAME	POSITION TITLE		
SHA, JIANG	Graduate Research Assistant		
EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.*)			
INSTITUTION AND LOCATION	DEGREE (*if applicable*)	YEAR(s)	FIELD OF STUDY
Peking University, Beijing, China	B.S.	1997-2001	Biology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

Research and Professional Experience:

2000 - 2001 Institution of Biophysics, Chinese Academy of Science

2001 - 2002 Molecular Biology Program, The University of Utah, Laboratory Rotation

2002 - current Graduate Research Assistant, Department of Medicinal Chemistry, The University of Utah, Salt Lake City